Acta Poloniae Pharmaceutica – Drug Research, Vol. 74 No. 6 pp. 1729–1738, 2017 ISSN 0001-6837
Polish Pharmaceutical Society

EVALUATION OF ANTIARRYTHMIC ACTIVITY OF NOVEL IMIDAZO[2,1-*F*]PURINE-2,4-DIONE AND IMIDAZOLIDINE-2,4-DIONE DERIVATIVES WITH AMINOALKYL MOIETIES

AGNIESZKA ZAGÓRSKA[1]*, ANNA CZOPEK[1], GRAŻYNA CHŁOŃ-RZEPA[1], MACIEJ PAWŁOWSKI[1], AGATA SIWEK[2], MAREK BEDNARSKI[3], MAŁGORZATA ZYGMUNT[3] and JACEK SAPA[3]

[1]Department of Medicinal Chemistry, [2]Department of Pharmacobiology, [3]Department of Pharmacological Screening, Chair of Pharmacodynamics, Jagiellonian University Medical College, 9 Medyczna Street, 30-688 Kraków, Poland

Abstract: The main goal of this study was to assess antiarrhythmic activity of novel aminoalkyl derivatives of imidazo[2,1-*f*]purine-2,4-dione and imidazolidine-2,4-dione exerting α_1 and 5-HT_{1A} receptors affinity. Tested compounds produced prophylactic and therapeutic antiarrhythmic activity in an adrenaline-induced model of arrhythmia. The strongest antiarrhythmic activity as well as the highest α_1-adrenoreceptor affinity (K_i = 13.9 nM) was found for 5-methyl-5-phenyl-3-[3-(4-(2-methoxyphenyl)piperazin-1-yl)propyl]-imidazolidine-2,4-dione (**12**). The results indicated a correlation between α_1-adrenoreceptor affinities and antiarrhythmic activity.

Keywords: α_1-adrenoreceptors, 5-HT_{1A}-receptors, imidazo[2,1-*f*]purine-2,4-dione, imidazolidine-2,4-dione, antiarrhythmic

The adrenergic system is a part of autonomic nervous system, which regulates neuronal, endocrine, metabolic and cardiovascular function. Adrenergic receptors (ARs), the members of G-protein coupled receptors (GPCRs) superfamily, are divided into three main classes α_1, α_2 and β. The α_1ARs are important mediators of sympathetic nervous system responses, particularly those involved in cardiovascular homeostasis *via* arteriolar smooth muscle constriction and cardiac contraction (1, 2). Moreover, α_1ARs have been implicated in the pathogenesis of cardiac hypertrophy, in ischemia-induced cardiac arrhythmias, and in ischemic preconditioning (3). Cardiac arrhythmias are cardiac rhythm disorders, which are significantly associated with increased risks of cardiovascular complications and sudden death. Atrial fibrillation (AF) is the most common sustained arrhythmia. The number of people suffering from this condition is expected to rise due to the gradual ageing of societies; therefore it seems to be an important epidemiological and public health issue. The role of α_1-ARs in various types of arrhythmia has been investigated even though α_1-adrenergic agents are not considered in the Vaughan-Williams classification of antiarrhythmic drugs. Some studies revealed the antiarrhythmic properties of α_1-adrenoreceptor antagonists in the case of ischemic arrhythmia (4, 5) and in this context, in recent decades, various α_1-adrenoreceptor antagonists have been designed and synthesized. A large group of adrenoreceptor antagonists are based on an arylpiperazinyl moiety, a well-known pharmacophore present in several classes of α_1-AR ligands. The large database of potent α_1-adrenoreceptor antagonists with the example of urapidil, WAY-100635, BMY-7378, REC-15/2739, RN5 (Fig. 1) allowed to evaluate pharmacophore models of the α_1-antagonist (6-15). Among them, Barbaro's model, especially useful for phenylpiperazine derivatives, has postulated five pharmacophore features: a positive ionizable atom (PI), three hydrophobic regions (HY1–HY3), and a hydrogen bond acceptor (HBA) (14). The above-mentioned α_1-adrenoreceptor antagonists possess an ortho-substituted phenyl ring corresponding to PI atom and both HY1 and HY2 regions, respectively. These compounds also

* Corresponding author: e-mail: agnieszka.zagorska@uj.edu.pl

contain an additional or fused aromatic moiety, terminating the heterocyclic fragments, which can correspond to the HY3-feature and they possess an alkyl spacer of 2-7 carbons (14). Derivatives of this type, called long-chain arylpiperazines (LCAPs) form a widely explored class of bioactive compounds, which is represented among others by the group of the $5HT_{1A}$ receptor ligands. The structural features of LCAPs, which determine selectivity for α_1-adrenergic receptors over serotonin 5-HT_{1A} are not clear, due to a high degree of similarity in amino acid sequence of receptors.

On the other hand, case reports (16-19) suggest that endogenous adenosine may cause clinically significant arrhythmias in patients during acute myocardial infarction, the sick sinus syndrome, car-

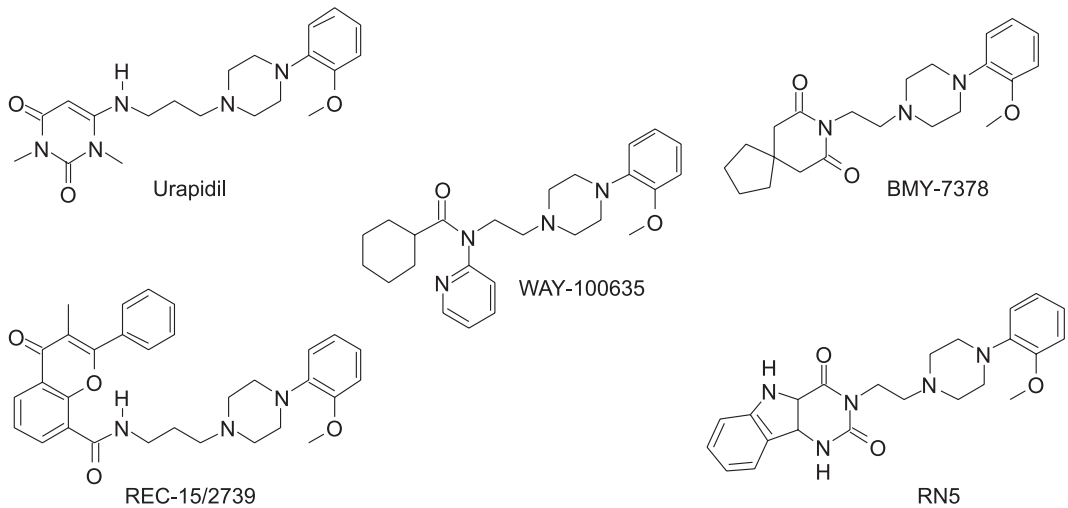
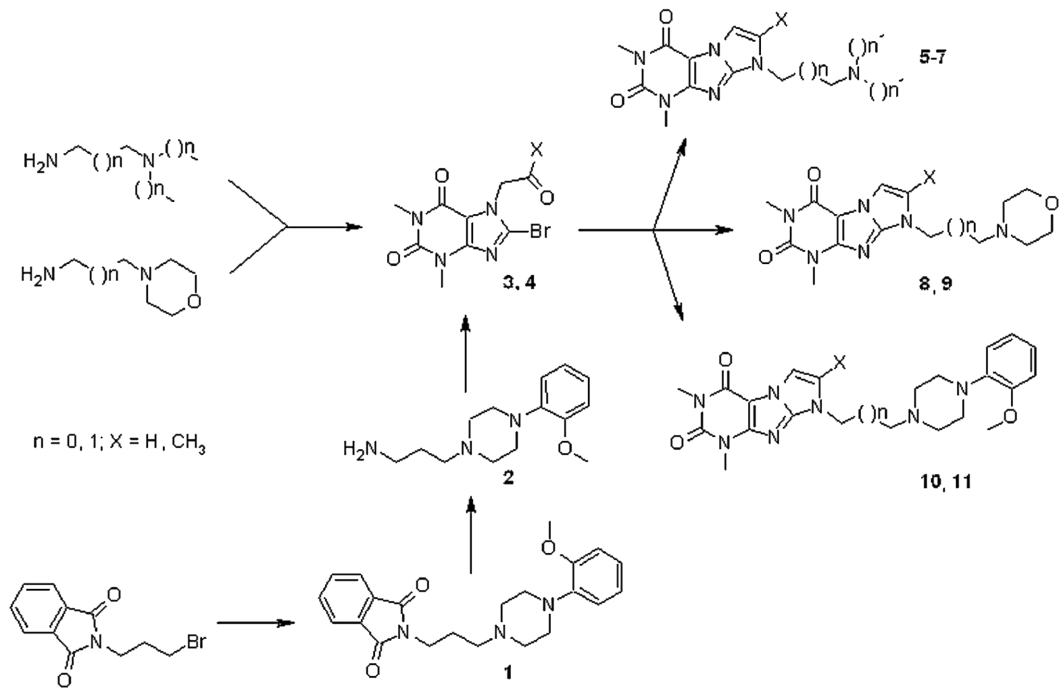


Figure 1. Chemical structures of potent α_1-AR antagonists



Scheme 1. Synthetic pathways of novel 1,3-dimethyl-(1*H*,8*H*)-imidazo[2,1-*f*]purine-2,4-dione derivatives (**5**-**11**).

diac arrest or cardiac transplant rejection. Based on this fact adenosine receptors antagonists appear to be effective in converting these arrhythmias to normal sinus rhythm (20). A large number of antagonists of the adenosine receptors have been developed in the group of xanthines, derived from the natural alkaloids theophylline and caffeine.

Our previous studies were focused on cardiovascular activity of N-hydroxyamide, alkanoloamides, dialkylamino-alkylamides and some of morpholinylalkylamides of pyrimidin-8-on[2,1-*f*]theophylline-9-alkylcarboxylic acids (21). The amide derivatives of alkylcarboxylic acids are significantly represented in a group of drugs, used in the treatment of cardiovascular disorders. These compounds have been most frequently presented as antiarrhythmic agents, which belong to I class Vaughan-William's classification. Some derivatives of pyrimidin-8-on[2,1-*f*]theophylline-9-alkylcarboxylic acids presented effects on arrhythmia and blood pressure parameters. The most interesting were dialkylamino-alkylamides and their morpholine analogs, which protected the heart against the bradycardia and diminished mortality of animals (21).

Moreover, we carried out chemical modification of phenytoin, a known anticonvulsant and antiarrhythmic agent, in order to find out which structural features influence on the antiarrhythmic potency and decrease its anticonvulsant action (22). Phenytoin (5,5-diphenyl-hydantoin) possessed structural properties similar to known α_1-AR antagonists but unlike them the selectivity of hydantoin derivatives with LCAPs moiety (4, 23, 24) for 5-HT_{1A} receptor and α_1-AR depends on the chain length of the alkyl spacer between hydantoin and piperazine and on the size of various substituents in hydantoin core.

On the basis of our previous work (21, 22) and taking into account the Barbaro's pharmacophore model of the α_1-antagonist, this paper reports further structural modifications in the previously synthesized series of LCAPs, in particular the introduction of small alkylamine group into 1,3-dimethyl-(1*H,8H)*-imidazo[2,1-*f*]purine-2,4-dione fragment (Scheme 1). Moreover, the LCAPs derivatives, containing 2-methoxy-phenylpiperazine moiety (structural element of urapidil), were designed in order to gain a new insight into the structural requirements that directly affect antiarrhythmic activity. Furthermore, to determine the role of the terminal heterocyclic moiety in selectivity and affinity for α_1-adrenergic *versus* 5-HT_{1A} receptors, the analogues with imidazolidine-2,4-dione (hydantoin) moiety, instead of imidazo[2,1-*f*]purine-2,4-dione system, were resynthesized (26). The main goal of the studies was to assess antiarrhythmic activity of the selected compounds which may display significant affinity for α_1-adrenergic and serotonin 5-HT_{1A} receptors.

EXPERIMENTAL

Chemistry

Structures of the investigated compounds (**5-11**) and their syntheses are presented in Scheme 1. The final derivatives of 1,3-dimethyl-(1*H,8H)*-imidazo[2,1-*f*]purine-2,4-dione were obtained within a reaction of cyclocondensation of 7-acetonyl-8-bromotheophylline (**1**) with appropriate alkylamine according to the previously reported method (25). 3-Bromopropyl-5-phenyl-5-alkyl-imidazolidine-2,4-dione derivatives were condensed with 2-methoxyphenyl-piperazine yielding the final compounds **12-13** (26). For the purpose of pharmacological evaluation, all the final compounds were converted into water-soluble hydrochloride salts. The structure elucidations of the newly synthesized compounds were carried out using different spectroscopic techniques including ^{1}H NMR and LC/MS. Further confirmations of the compounds were carried out by elemental analysis (± 0.4%). The elemental analysis data and some physical properties of these compounds were reported in the experimental part.

All the chemicals used were commercial products employed without purification. Purity of the synthesized compounds was confirmed by TLC performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany) with the following solvents: benzene/acetone/methanol (10 : 10 :10). Spots were detected by their absorption under UV light (λ = 254 nm). ^{1}H NMR spectra were recorded on Varian BB 200 spectrometer at 300 MHz, using TMS (0.00 ppm) and chloroform-d_1, *J* values are in hertz (Hz), and splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), m (multiplet). The LC/MS system consisted of a Waters Acquity UPLC, coupled to Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All analyses were carried out using an Acquity UPLC BEH C18, 50 × 2.1 mm reversed-phase column. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 100 to 700 m/z; 8 scans were summed up to produce the final spectrum. Elemental analyses were found within ± 0.4% of the theoretical values. Melting points (m.p.) were determined with a Büchi

apparatus and are uncorrected. Column chromatography separations were carried out on column with Merck Kieselgel 60 using the solvents: dichloromethane/methanol (90 : 10).

General procedures for preparation of intermediate compounds (1-4)

N-aminopropyl derivatives of 2-metoxyphenylpiperazine (**2**) were obtained according to Gabriel synthesis of primary alkyl amines in two-step procedure with slight modification (25). Firstly, 2-metoxy-phenylpiperazine was alkylated with the N-bromopropylphthalimide (**1**). Then, after hydrazinolysis of 2-1H-isoindole-1,3(2H)-dione, the proper primary amine was obtained (**2**). 7-Acetic-8-bromotheophylline aldehyde (**3**) was obtained according to the previously described procedure (25). 8-Acetonyl-8-bromotheophylline (**4**) was obtained in the reaction of alkylation of 8-bromotheophylline (1 eq) with chloro-2-propanone (1.1 eq) in boiling acetonitrile, in the presence of K_2CO_3 (3 eq). After completion, the precipitate was recrystallized from absolute ethanol. The results of spectral (^{1}H NMR) and elemental analysis confirmed the identity of the compound with obtained by other methods.

General procedures for preparation of final compounds (5-13)

Mixtures of 7-acetonyl-8-bromotheophylline with a 2-fold excess of appropriate amine in 2-metoxyethanol, were heated under reflux for 24 h. After evaporation of the solvent to the brown oil residues, products were separated by column chromatography.

*C*ompounds **10-13** were resynthesized in a scale of 100 mg and analytical data for this compounds, were consistent with previously published ones (25, 26).

7-Methyl-8-[2-N,N-dimetyl-ethyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (5)

Yield 87%; m.p. 183-187^{o}C, Rf = 0.27 (A);^{1}H-NMR ($CDCl_3$, δ, ppm): 2.29 (s, 3H,C*H*$_3$), 2.33 (ds, *J* = 1.1 Hz, 6H, N(CH_3)$_2$), 2.71 (t, *J* = 13.4 Hz, 2H, $CH_2CH_2N(CH_3)_2$), 3.42 (s, 3H, N3-CH_3), 3.58 (s, 3H, N1-CH_3), 4.07 (t, *J* = 13.4 Hz, 2H, $CH_2CH_2N(CH_3)_2$), 7.13 (s, 1H, C6*H*); ESI-MS (*m*/*z*) 305.35 (M + H)$^+$: Analysis: calcd. for $C_{14}H_{20}N_6O_2$: C, 55.25; H, 6.62; N, 27.61%; found: C, 55.24; H, 6.63; N, 27.54%.

7-Methyl-8-[2-N,N-dimetyl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (6)

Yield 69%, m.p. 207-209^{o}C; Rf = 0.32 (A); ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.29 (s, 3H,C*H*$_3$), 2.33 (ds, *J* = 1.1 Hz, 6H, N(CH_3)$_2$), 2.71 (t, *J* = 13.4 Hz, 2H, $CH_2CH_2N(CH_3)_2$), 3.42 (s, 3H, N3-CH_3), 3.58 (s, 3H, N1-CH_3), 4.07 (t, *J* = 13.4 Hz, 2H, $CH_2CH_2N(CH_3)_2$), 7.13 (s, 1H, C6*H*); ESI-MS (*m*/*z*) 319.35 (M + H)$^+$: Analysis: calcd. for $C_{15}H_{22}N_6O_2$: C, 56.59; H, 6.96; N, 26.40; found: C, 56.24; H, 6.93; N, 26.54%.

7-Methyl-8-[2-N,N-diethyl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (7)

Yield 80%; m.p. 205-204^{o}C, Rf = 0.38 (A); ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.48-1.63 (m, 2H, $CH_2CH_2CH_2$), 2.39 (s, 3H,CH_3), 2.51-2.63 (m, 2H, $CH_2CH_2CH_2$), 3.00-3.25 (m, 10H, N(C_2H_5)$_2$), 3.43 (s, 3H, N3-CH_3), 3.56 (s, 3H, N1-CH_3), 4.17 (t, *J* = 13.4 Hz, 2H, $CH_2CH_2CH_2$), 7.21 (s, 1H, CH); ESI-MS (*m*/*z*): 347.43 (M + H)$^+$; Analysis: calcd. for $C_{17}H_{26}N_6O_2$: C, 58.94; H, 7.56; N, 26.26; found: C, 58.97; H, 7.33; N, 26.20%.

7-Methyl-8-[2-N-morpholinyl-ethyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (8)

Yield 87%; m.p. 195-196^{o}C; R_f = 0.55 (A); ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.34 (s, 3H, C*H*$_3$), 2.50-2.53 (m, 4H, N(CH_2)$_2$), 2.7 (t, *J* = 12.9 Hz, 2H, CH_2CH_2), 3.42 (s, 3H, N3-CH_3), 3.58 (s, 3H, N1-CH_3), 3.63-3.66 (m, 4H, (CH_2)$_2$O), 4.08 (t, *J* = 12.65 Hz, 2H, CH_2CH_2), 7.15 (s, 1H, CH) ESI-MS (*m*/*z*) 347.38 (M + H)$^+$; Analysis: calcd. for $C_{16}H_{22}N_6O_3$: C, 55.48; H, 6.40; N, 26.24; found: C, 55.57; H, 6.40; N, 24.42%.

7-Methyl-8-[2-N-morpholinyl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (9)

Yield 92%; m.p. 147-149^{o}C; R_f = 0.52 (A); ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.00-2.09 (m, 2H, $CH_2CH_2CH_2$), 2.33-2.41 (m, 9H, $CH_2N(CH_2)_2$ + CH_3), 3.41 (s, 3H, N3-CH_3), 3.57 (s, 3H, N1-CH_3), 3.66-3.69 (m, 4H, (CH_2)$_2$O), 4.05 (t, *J* = 12.65 Hz, 2H, C*H*$_2$CH$_2$CH$_2$), 7.13 (s, 1H, CH) ESI-MS (*m*/*z*) 361.41 (M + H)$^+$, Analysis: calcd. for $C_{17}H_{24}N_6O_3$: C, 56.65; H, 6.71; N, 23.31; found: C, 56.57; H, 6.70; N, 23.18%.

7-Methyl-8-[3-(N4-2'-methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (10)

Yield 85%, m.p. 195-196^{o}C, R_f = 0.66 (A) ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.11-2.16 (m, 2H, $CH_2CH_2CH_2$), 2.39 (s, 3H, 7CH_3) 2.39-2.47 (t, *J* = 13.3 Hz, 2H, $CH_2CH_2CH_2$), 2.49-2.67 (m, 4H, (CH_2)$_2$N1), 3.05-3.1 (m, 4H, N4(CH_2)$_2$), 3.46 (s, 3H, N3CH_3), 3.63 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.12-4.16 (t, *J* = 13.7 Hz, 2H, N8CH_2), 6.88-7.07 (m, 4H, H), 7.18 (s, 1H, C6*H*). ESI-MS (*m*/*z*) 466.5 (M + H)$^+$. Analysis: calcd. for $C_{24}H_{31}N_7O_3$: C, 61.9;

H, 6.71; N, 21.06; found: C, 61.72; H, 6.56; N, 20.82%.

8-[3-(N4-2'-Methoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione (11)

Yield 73%, m.p. 230-231°C, R_f = 0.67 (A) ^{1}H-NMR ($CDCl_3$, δ, ppm): 2.12-2.17 (m, 2H, $CH_2CH_2CH_2$), 2.45-2.48 (t, *J* = 13.7 Hz, 2H, $CH_2CH_2CH_2$), 2.50-2.68 (m, 4H, $(CH_2)_2$N1), 3.13-3.20 (m, 4H, N4$(CH_2)_2$), 3.47 (s, 3H, N3CH_3), 3.64 (s, 3H, N1CH_3), 3.89 (s, 3H, OCH_3), 4.19-4.23 (t, *J* = 13.7 Hz, 2H, N8CH_2), 6.87-7.07 (m, 5H, H+C7H), 7.43-7.44 (d, *J* = 2.4 Hz, 1H, C6H). ESI-MS (*m*/*z*) 452.5 ($M + H^+$), Analysis: calcd. for $C_{23}H_{29}N_7O_3$: C, 61.18; H, 6.47; N, 21.71; found: C, 60.88; H, 6.51; N, 21.72%.

5-Methyl-5-phenyl-3-[3-(4-(2-methoxyphenyl) piperazin-1-yl)propyl]-imidazolidine-2,4-dione (12)

Yield: 46%; m.p. 156–159°C; ^{1}H NMR (300 MHz, ($CDCl_3$, δ, ppm): 1.89–1.80 (m, 5H), 2.43–2.35 (t, *J* = 7 Hz, 2H), 2.62–2.54 (m, 4H), 3.10–3.01 (m, 4H), 3.64–3.55 (t, *J* = 7 Hz, 2H), 3.88–3.82 (s, 3H), 5.56 (s, 1H), 7.03–6.81 (m, 4H), 7.51–7.33 (m, 5H). Analysis: calcd. for $C_{24}H_{30}N_4O_3$: C, 68.22; H, 7.16; N, 13.26; found: C, 67.70; H, 7.24; N, 13.15%.

5-Cyclopropyl-5-phenyl-3-[3-(4-(2-methoxyphenyl) piperazin-1-yl)propyl]-imidazolidine-2,4-dione (13)

Yield: 49%; M.p. 148–150°C; ^{1}H NMR (300 MHz, ($CDCl_3$, δ, ppm): 0.76–0.29 (m, 4H), 1.75–1.64 (m, 1H), 1.91–1.80 (t, *J* = 7 Hz, 2H), 2.47–2.39 (m, 2H), 2.66–2.58 (m, 4H), 3.11–3.02 (m, 4H), 3.62–3.56 (t, *J* = 7 Hz, 2H), 3.87–3.83 (s, 3H), 6.01 (s, 1H), 7.02–6.82 (m, 4H), 7.60–7.32 (m, 5H). Anal. calcd for $C_{26}H_{32}N_4O_3$: C, 69.62; H, 7.19; N, 12.49. Found: C, 69.98; H, 7.34; N, 12.67.

Pharmacology

***In vitro* binding assay**

Determination of the affinity of the tested compounds at the α_1-adrenoreceptors

The affinity of the obtained compounds (**5**-**13**) was evaluated by radioligand binding assays (the ability to displace [^{3}H]-prazosin from α_1) on rat cerebral cortex. The tissue was homogenized in 20 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.6 using an Ultra Turrax T25B (IKA) homogenizer. The homogenate was centrifuged at 20 000 × *g* for 20 min. The resulting supernatant was decanted and pellet was resuspended in the same buffer and centrifuged again in the same conditions. The final pellet was resuspended in appropriate volume of buffer (10 mg/1 mL). [^{3}H]prazosin (spec. act. 85 Ci/mM, Perkin Elmer) was used for labeling α_1-receptor. 240 mL of the tissue suspension, 30 mL of 10 μM phentolamine (displacer), 30 mL of 0.2 nM [^{3}H]Prazosin and 30 mL of the analyzed compound were incubated at 30°C for 30 min. The concentrations of analyzed compounds ranged from 10^{-10} to 10^{-5} M. The incubation was terminated by rapid filtration over glass fiber filters FilterMate B (PerkinElmer, USA) using 96-well FilterMate harvester (PerkinElmer, USA). Five rapid washes were performed with ice-cold 50 mM Tris-HCl buffer, pH 7.6. Filter mate was dried in microwave and placed in plastic bag (PerkinElmer, USA) and soaked in 10 mL of liquid scintillation cocktail Ultima Gold MV (PerkinElmer, USA). After even distribution of scintillation cocktail filter bag was sealed. The radioactivity on the filter was measured in MicroBeta TriLux 1450 scintillation counter (PerkinElmer, USA). All the assays were made in duplicate.

Determination of the affinity of the tested compounds at the 5-HT_{1A} receptor

One millimolar stock solutions of the compounds to be tested were prepared in DMSO. Serial dilutions of compounds were prepared in 96-well microplates in assay buffers using an automated pipetting system (epMotion 5070; Eppendorf). Radioligand binding was performed using cryopreserved membranes from cells stably expressing the relevant human receptor. Reagents and condition: 50 μL working solution of the tested compounds, 50 μL radioligand solution and 150 μL diluted membranes prepared in assay buffer were transferred to 96-well microplates. These were covered with sealing tape, mixed and incubated. The reaction was terminated by rapid filtration through UniFilter 96 GF/B filter microplate and ten rapid washes with 200 μL 50 mM Tris buffer (4°C, pH 7.4) were performed using vacuum manifold and 96-well pipettor. The UniFilter microplates were dried overnight at 37°C in dry incubator. The UniFilter bottoms were sealed and 30 μL of liquid scintillator Betaplate Scint (Perkin Elmer) was added to each well. The plates were allowed to equilibrate for 1 h and then radioactivity was counted in MicroBeta TriLux 1450 scintillation counter (PerkinElmer) at approximately 30% efficiency. Data were fitted to a one-site curve-fitting equation with Prism 5 (GraphPad Software) and K_i values were calculated using the Cheng-Prusoff equation (27). Each compound was tested in eight concentrations from 1 × 10^{-4} M to 1 × 10^{-11} M (final concentration). All the assays were carried out in duplicates (n = 2).

***In vivo* assay**

Animals

The experiment was carried out on male Wistar rats (180-220 g). The animals were housed in constant temperature facilities exposed to 12 : 12 light-dark cycle and maintained on a standard pellet diet and tap water was given *ad libitum*. Control and experimental groups consisted of 6-8 animals each. The investigated compounds were administered intravenously (*i.v.*) in a form of a suspension in 0.9% physiological saline. Control animals received the equivalent volume of physiological saline. All procedures were conducted according to guidelines of ICLAS (International Council on Laboratory Animals Science) and approved by The Local Ethic Committee on Animal Experimentation.

The effect on blood pressure response to methoxamine

Male Wistar normotensive rats were anesthetized with thiopental (75 mg/kg, *ip*). The right carotid artery was cannulated with polyethylene tube filled with heparin in saline to facilitate pressure measurement using the Datamax apparatus (Columbus Instruments). The tested compounds were injected in a single dose of 0.5 mg/kg into the caudal vein after a 5 min stabilization period.

Prophylactic antiarrhythmic activity in adrenaline-induced arrhythmia

Arrhythmia was evoked in rats anesthetized with thiopental (60 mg/kg, *ip*) by an *iv* injection of adrenaline (20 µg/kg, in volume of 1 mL/kg) according to the method of Szekeres (28). The tested compounds were administered at the doses of 0.25, 0.5, 1 mg/kg *iv* 15 min before the administration of adrenaline. The criteria for antiarrhythmic activity were the lack of premature beats and the inhibition of cardiac arrhythmia compared to the control group.

Therapeutic antiarrhythmic activity in adrenaline-induced arrhythmia

Therapeutic antiarrhythmic activity was determined according to the method of Szekeres (28). The arrhythmia was evoked in rats under anesthesia with thiopental (60 mg/kg, *ip)* by *iv* injection of adrenaline (20 µg/kg, in volume of 1 mL/kg). The tested compounds were administered at the doses of 0.25, 0.5, 1 mg/kg by *iv* route at the peak of arrhythmia, immediately after administration of adrenaline. The ECG was recorded continuously for 5 min. The criterion of antiarrhythmic activity was the reduction of premature beats in comparison to the control group.

The effect on normal electrocardiogram (ECG)

Electrocardiographic investigations were performed using a Multicard 30 apparatus with standard lead II and a paper speed of 50 mm/s. The tested compounds were administered intravenously (*iv*) at a dose of 1 mg/kg. The ECG was recorded just before and 1, 5 and 15 min after the administration of compounds.

Statistical analysis

The data were evaluated by one-way analysis of variance (ANOVA) followed by Duncan test. The difference of means was statistically significant if $p < 0.05$.

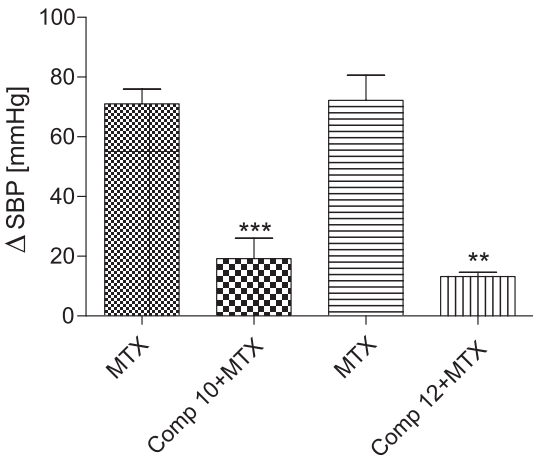


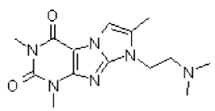
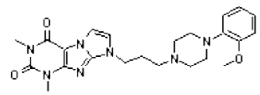
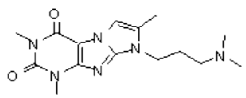
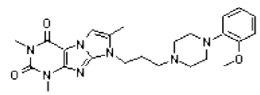
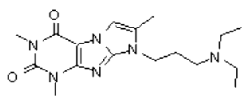
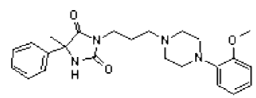
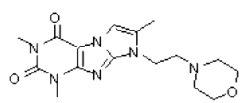
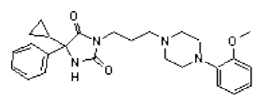
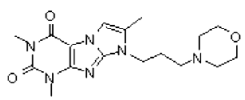
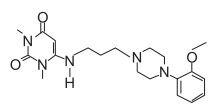
Figure 2. The effect of compounds **10** and **12** on blood pressure response to methoxamine (**p < 0.01, *** p < 0.001)

RESULTS

According to the aim of this study two series of 1,3-dimethyl-(1H,8H)-imidazo[2,1-*f*]purine-2,4-dione and 5,5-disubstituted imidazolidine-2,4-dione derivatives were obtained and evaluated for their α_1 and 5-HT_{1A} affinity by standard competitive displacement assays The affinity data (K_i) of tested compounds are shown in Table 1.

The *in vitro* radioligand binding studies showed that derivatives of 1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione with alkylaminealkyl or morpholinylalkyl moieties (**5**-**9**) did not bind at α_1 recognition sites. All of the 2-methoxyphenyl-piperazinyl-propyl derivatives of 1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione and 5,5-disubstituted imidazolidine-2,4-dione (**10**-**13**) were potent α_1 receptor ligands with K_i within the range of 13.9-36.7 nM. Based on this

Table 1. The chemical structures of final compounds (5-13) and their affinity towards α_1-adrenergic and 5-HT_{1A} receptors.

Compound no.	Chemical structures	K_i [nM]		Compound no.	Chemical structures	K_i [nM]	
		α_1	5-HT_{1A}			α_1	5-HT_{1A}
5		> 1000	> 1000	**10**		19.3 ± 3.4	25.4 ± 6.7*
6		> 1000	> 1000	**11**		36.7 ± 1.6	16.8 ± 7.6*
7		> 1000	> 1000	**12**		13.9 ± 1.3	47.0 ± 1.0*
8		> 1000	> 1000	**13**		15.3 ± 1.4	11.0 ± 1.0*
9		> 1000	> 1000	Urapidil		6.9 ± 0.05	6.4**

*data from (25, 26), **pIC_{50} value.

results, the representatives of both group of compounds **10** and **12**, with the highest affinity for α_1 receptors, were selected for further pharmacological *in vivo* evaluation and their influence on the pressor responses to methoxamine were studied. It is generally accepted that α_1-ARs antagonists diminish the pressor response to methoxamine (α_1-ARs agonists). Methoxamine given *iv* to rats caused a pressor response at the dose 150 mg/kg. Compounds 10 and **12** given *iv* in doses of 0.5 mg/kg antagonized the pressor response elicited by methoxamine (Fig. 2), thereby exhibited α_1-antagonistic properties.

The newly synthesized aminoalkyl derivatives of 1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione (**5**-**9**) did not show affinity for serotonin 5-HT_{1A} receptor (Table 1). On the contrary, compounds derived from 1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione with 2-methoxyphenyl-piperazinyl-propyl moiety (**10**, **11**) displayed high affinity for 5-HT_{1A} receptors (Table 1) (25). Furthermore, their counterparts with imidazolidine-2,4-dione core (**12**, **13**) were also reported as potent 5-HT_{1A} receptor ligands (26).

The selected compounds **10** and **12** with α_1-antagonistic activity were further evaluated for their antiarrhythmic properties in adrenaline-induced arrhythmia. These compounds (**10**, **12**) injected intravenously 15 min before adrenaline, diminished the occurrence of heart-rhythm disturbances. In the control group the occurrence of ventricular premature beats (VBs) and blocks incidence was 100%. All tested compounds diminished the incidence of arrhythmia in comparison to the control. Compound **10** diminished the incidence of ventricular premature beats by 50-33%. Among studied compounds, the most potent prophylactic antiarrhythmic effect was produced by compound **12**, which significantly reduced the VBs and blocks incidence of 100% both at a dose of 0.5 and 1.0 mg/kg (Table 2). In therapeutic model of adrenaline-induced arrhythmia, the compounds **10** and **12** administered intravenously at the peak of arrhythmia prevented and/or reduced the number of premature ventricular beats, blocks and mortality (Table 3). The most potent antiarrhythmic effect was produced by compound **12**, which significantly reduced the incidence of: ventricular prema-

Table 2. Prophylactic anthiarrhytmic activity in adrenaline-induced arrythmia.

Compound no.	Dose [mg/kg]	Ventricular premature beats incidence (%)	Bigeminy incidence (%)	Blocks incidence (%)	Mortality (%)
Control	-	100	22	100	70
10	0.5	33	17	0	0
	1	50	0	0	0
12	0.5	0	0	0	0
	1	0	0	0	0
Urapidil	0.5	67	0	100	0
	1	50	0	50	0

Table 3. Therapeutic antiarrhythmic activity in adrenaline-induced arrhythmia.

Compound no.	Dose [mg/kg]	Ventricular premature beats incidence (%)	Bigeminy incidence (%)	Blocks incidence (%)	Mortality (%)
Control	-	100	20	100	64
10	0.25	80	20	100	50
	0.5	50	0	0	50
	1	60	0	0	50
12	0.06	60	0	40	20
	0.125	33	0	80	0
	0.5	20	0	0	0

ture beats and mortality at a dose 0,125 mg/kg, (Table 3).

The ECG experiments showed that compounds **10** and **12** did not significantly effect on the normal ECG in anesthetized rats after 15 min after administration (Table 4). They did not significantly influence intervals PR, QRS and QT, as well as the heart rate after intravenous administration.

DISCUSSION AND CONCLUSION

The pharmacological *in vitro* activity of the investigated compounds (**5**-**13**) toward α_1 and 5-HT_{1A} receptors depends mostly on the amine fragment. Introduction of dimethylamine (**5**, **6**), diethylamine (**7**) and morpholine (**8**, **9**) connected with imidazo[2,1-*f*]purine-2,4-dione by 2-3 carbon spacer, did not result in significant affinity for α_1 as well as for 5-HT_{1A} receptors. The exchange of alkyl amine substituents for 2-methoxyphenylpiperazine moiety (**10** and **11**) induced affinity for scheduled receptors. The analysis of the impact of the substituent in position 7 of imidazo[2,1-*f*]theophylline, has shown that the methyl group only slightly decreased affinity for α_1-adrenergic receptor (**10** *vs*. **11**). The replacement of heterocyclic fragment, which can correspond to the HY3-feature of Barbaro's model, from imidazo[2,1-*f*]theophylline to imidazolidine-2,4-dione, caused no significant changes in receptor activity profile (Table 1). Moreover, it could be expected that compounds with LCAPs moiety might displayed dual activity for α_1-AR and 5-HT_{1A}R, and indeed compounds with 2-methoxyphenyl-piperazinylpropyl fragment (**10**-**13**) showed significant affinity for α_1-adrenergic and serotonin 5-HT_{1A} receptors. It is noteworthy that among the most active structures, compound **12** exhibited some preference towards α_1-adrenergic receptors, whereas compound **11** slightly stronger interacted with 5-HT_{1A} receptor site.

In the next step of the pharmacological evaluation, the selected compounds **10** and **12** as the representatives of both heterocyclic system with the highest α_1-adrenergic receptor affinity and α_1-adrenolytic properties were evaluated *in vivo* for their antiarrhythmic activity in adrenaline-induced arrhythmia model.

In prophylactic model of adrenaline-induced arrhythmia, rapid intravenous injection of adrenaline caused reflex bradycardia (100%), atrioventricular disturbances, extrasystoles (100%), which led to the death of approximately 70% of animals of the control group within 15 min of the observation. The selected compounds were more active in all experiments than reference drug urapidil, especially compound **12**, which protected 100% of animals. It is worth noting, that tested compounds **10** and **12** demonstrated prominent antiarrhythmic activity in both prophylactic (given *iv* 15 min before arrhythmogen) and therapeutic (given *iv* at the peak of arrhythmia) models of adrenaline-induced arrhythmia. The observed profile of activity allows application of such agents not only in prevention, but also in treatment of acute episodes of arrhythmia. Moreover, the observed lack of change in the length of the Q-T interval in ECG experiments, showed that compounds **10** and **12** did not show potential proarrhythmic activity.

The tested compounds **10** and **12** as well as the reference drug – urapidil (6-({3-[4-(2-methoxyphenyl)piperazin-1-yl]propyl}amino)-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione) belong to the class of LCAPs derivatives. From the pharmacological point of view, urapidil as α_1-adrenolytic, causes a fall in blood pressure and prevents the expected reflex rise in sympathetic nerve activity. In the previous studies (30-32), in concentration of 10^{-6}M, urapidil significantly diminished the occurrence of ventricular tachycardia and ventricular fibrillation in the isolated rat heart. Moreover, urapidil revealed also potent prophylactic and therapeutic antiarrhythmic effect in adrenaline-induced arrhythmia after intravenous administration with ED_{50} values 1.26 and 3.4 mg/kg, respectively.

The pharmacological results of novel aminoalkyl derivatives with imidazo[2,1-*f*]purine-2,4-dione (**10**) and imidazolidine-2,4-dione (**12**) cores suggest that those antiarrhythmic activity may be related to their α_1-adrenolytic properties.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgment

This study was partially funded by National Science Center grant (No. UMO-2012/07/B/NZ7/01173).

REFERENCES

1. Woodcock E.A., Du X.J., Reichelt M.E., Graham R.M.: Cardiovasc. Res. 77, 452 (2008).
2. Graham R.M.: Cleve. Clin. J. Med. 57, 481 (1990).
3. Mitchell M.B., Meng X., Ao L., Brown J.M., Harken A.H., Banerjee A.: Circ. Res. 76, 73 (1995).

4. Handzlik J., Szymanska E., Wojcik R., Dela A., Jastrzebska-Wiesek M., et al.: Bioorg. Med. Chem. 20, 4245 (2012).
5. Handzlik J., Bajda M., Zygmunt M., Maciag D., Dybala M., et al.: Bioorg. Med. Chem. 20, 2290 (2012).
6. Bremner J.H., Coban B., Griffith R., Groenewoud K.M., Yates B.F.: Bioorg. Med. Chem. 8, 201 (2000).
7. Romeo G., Materia L., Marucci G., Modica M., Pittala V., et al.: Bioorg. Med. Chem. Lett. 16, 6200 (2006).
8. Betti L., Botta M., Corelli F., Floridi M., Giannaccini G., et al.: J. Med. Chem. 45, 3603 (2002).
9. Betti L., Botta M., Corelli F., Floridi M., Fossa P., et al.: Bioorg. Med. Chem. Lett. 12, 437 (2002).
10. Betti L., Floridi M., Giannaccini G., Manetti F., Strappaghetti G., et al.: Bioorg. Med. Chem. Lett. 13, 171 (2003).
11. Betti L., Corelli F., Floridi M., Giannaccini G., Maccari L., et al.: J. Med. Chem. 46, 3555 (2003).
12. Barbaro R., Betti L., Botta M., Corelli F., Giannaccini G., et al.: J. Med. Chem. 44, 2118 (2001).
13. Romeo G., Russo F., Guccione S., Barbarulo D., De Blasi A.: Farmaco 50, 471 (1995).
14. Barbaro R., Betti L., Botta M., Corelli F., Giannaccini G., et al.: Bioorg. Med. Chem. 10, 361 (2002).
15. Kuo G.H., Prouty C., Murray W.V., Pulito V., Jolliffe L., et al.: Bioorg. Med. Chem. 8, 2263 (2000).
16. Wilbur S.L., Francis E Marchlinski F.E.: Am. J. Cardiol. 79, 30 (1997).
17. Llach A., Molina C.E., Prat-Vidal C., Fernandes J., Casadó V., et al.: Eur. Heart J. 32, 721 (2011).
18. Strickberger S.A., Man K.C., Daoud E.G., Goyal R., Brinkman K. et al.: Ann. Intern. Med. 127, 417 (1997).
19. Pelter M.M., Carey M.G., Kasmani R., Irani F.: Am. J. Crit. Care 19, 189 (2010).
20. Bertolet B.D., Hill J.A., Belardinelli L.: J. Am. Coll. Cardiol. 25, 421 (1995).
21. Zagórska A., Fhid O., Pawlowski M., Filipek B., Zygmunt M.: Lett. Drug Des. Discov. 3, 346 (2006).
22. Czopek A., Byrtus B., Zagórska A. , Siwek A., Kazek G., et al.: Pharmacol. Rep. 68, 886 (2016).
23. Handzlik J., Maciag D., Kubacka M., Mogilski S., Filipek B., et al.: Bioorg. Med. Chem. 16, 5982 (2008).
24. López-Rodríguez M.L., Ayala D., Benhamú B., Morcillo M.J., Viso A.: Curr. Med. Chem. 9, 443 (2002).
25. Zagorska A., Jurczyk S., Pawlowski M., Dybala M., Nowak G., et al.: Eur. J. Med. Chem. 44, 4288 (2009).
26. Czopek A., Byrtus H., Kolaczkowski M., Pawlowski M., Dybala M., et al.: Eur. J. Med. Chem. 45, 1295 (2010).
27. Cheng Y., Prusoff W.: Biochem. Pharmacol. 22, 3099 (1973).
28. Szekeres L., Papp G.: Handbook of experimental pharmacology. Springer-Verlag, New York 1975.
29. Ramage A.G.: Br. J. Pharmacol. 102, 998 (1991).
30. Sapa J., Filipek B., Nowinski L.: Pharmacol. Rep. 63, 455 (2011).
31. Kubacka M., Mogilski S., Bednarski M., Razny K., Sapa J., et al.: Pharmacology 94, 287 (2014).
32. Wu K.S., Zhou J.C., Li H.Y., Gu D.Y., Pan K.H., et al.: J. Thorac. Dis. 6, 1765 (2014).

Received: 13. 10. 2016